



Mimosine facilitates Fe uptake by *Leucaena leucocephala* subsp. *glabrata* in alkaline soils by solubilizing Fe-oxides

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Abstract

Purpose Giant leucaena is a tropical tree-legume that thrives in alkaline and calcareous soils. It contains large amounts of mimosine, the physiological role of which is not well understood. The goals of this study were to determine i) if mimosine is released by giant leucaena into the rhizosphere ii) if mimosine enhances solubility of Fe present in various forms in alkaline soil, and iii) if mimosine enhances transition metal uptake in giant leucaena.

Methods Mimosine in the extracts of rhizosphere soil and root exudates was analyzed by high performance liquid chromatography (HPLC) and liquid chromatography/mass spectrometry (LC/MS) to determine if it is released by giant leucaena. Iron concentration in tissue and aqueous samples was determined by ICP-OES analysis.

Results Mimosine was detected in both the rhizosphere eluates and root exudates. Mimosine added to samples of goethite, hematite and FeCl₃ increased Fe solubility in water. Giant leucaena plants grown in

alkaline soils and supplied with mimosine had higher levels of chlorophyll, Fe and Mn than the control plants.

Conclusion Mimosine is released by giant leucaena roots into the rhizosphere, where it interacts with Fe. Mimosine enhances solubility of insoluble Fe-oxides and prevents conversion of ferric Fe to insoluble oxides at an alkaline pH. Mimosine enhances Fe and Mn uptake in plants growing in alkaline soil conditions.

Keywords Goethite · Hematite · Ferric · Ferrous · Fe-oxides · Phytochelator · Phytosiderophore

Introduction

Approximately 30% of the world's cultivated lands contain calcareous soils with high pH (Bashir et al. 2010). In aerobic and alkaline soils, Fe is sparingly soluble and as a consequence, plants growing under these conditions often exhibit symptoms of Fe deficiency (Marschner 1995; Ogo et al. 2011). Iron-deficient plants undergo leaf chlorosis, have stunted growth, and produce low yields. Iron is one of the most important soil micronutrients and is involved in many biological pathways such as photosynthesis, respiration and DNA synthesis (Rout and Sahoo 2015). Iron is required by plants at concentrations ranging from 10 nM to 1 mM; however, the amount of free ferric and ferrous Fe in soils is less than 10

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fM, which is too low for optimal plant growth and development (Guerinot and Yi 1994; Kim and Guerinot 2007; Marschner 1995). On the other hand, Fe is one of the most abundant elements in the earth comprising roughly 5.6% of the earth's crust (Morrissey and Guerinot 2009). Although it is highly abundant in the soil, Fe remains a limiting nutrient for plants due to poor its solubility and availability. In alkaline soils, ferrous ions are scarce and Fe is mostly present as insoluble ferric oxides such as goethite (FeOOH) and hematite (Fe₂O₃) (Wang et al. 2018). Both goethite and hematite are abundant in tropical environments (Journet et al. 2014).

To acquire Fe from the soil, plants utilize one of two strategies. Non-graminaceous plants utilize strategy I, while graminaceous plants utilize strategy II. In strategy I, plant roots secrete organic acids and natural chelates into the rhizosphere, where they bind to and help to solubilize soil Fe. Ferric reductase oxidase enzymes (FRO) also known as ferric chelate reductases, on the root surface, reduce the ferric-chelates formed in the rhizosphere to ferrous ions. Then, an Fe-regulated transporter (IRT) takes up the ferrous ions into the plants. In some strategy I-utilizing plants, protons are secreted by plant roots through H⁺ pumps (AHA). This serves to acidify the soil and helps to solubilize ferric ions, which can then bind to chelating compounds such as organic acids, which are also secreted by plants. In strategy II of Fe uptake from soils, Fe-binding compounds called phytosiderophores are secreted by the transporter of mugineic acid (TOM) efflux protein. One of these phytosiderophores is 2'-deoxymugineic acid (DMA), which binds to ferric ions in soils to form a ferric-DMA complex that is taken up by the yellow stripe-like family of peptide transporters (YSL). Some plants like rice and maize, possess proteins for both strategies of Fe uptake from the soil (Li et al. 2018; Wairich et al. 2019).

The tropical tree-legume *Leucaena leucocephala* (leucaena) thrives in alkaline and calcareous soils (Brewbaker 2016). Honda and Borthakur (2019) suggested that leucaena may possess dual strategies for Fe uptake from the soil. A number of strategy I and II genes were found to be expressed at high levels in roots, leaves, and stems of giant leucaena treated under various Fe availability conditions (Honda and Borthakur 2020). In the same study, the authors suggested that mimosine plays an important

role in both Fe-uptake strategies. Mimosine is a non-proteinogenic amino acid found in all parts of giant leucaena at various concentrations. Mimosine has been shown to bind various metallic cations at acidic, neutral and alkaline pHs (Honda and Borthakur 2020). Mimosine was also found to be a stress-response molecule that has various stress-alleviating roles and fluctuates in giant leucaena when exposed to different environmental stresses (Honda and Borthakur 2021). Mimosine shares structural similarities to other known phytochelatons such as caffeic acid, maltol and L-DOPA. Like mimosine, these compounds have various roles during plant stress.

The objective of the present study was to study the role of mimosine as a phytosiderophore in giant leucaena. Mimosine is hypothesized to facilitate Fe-uptake in giant leucaena from alkaline soils by solubilizing Fe-oxides. This hypothesis was tested by conducting experiments to (i) verify that mimosine is released into the rhizosphere; (ii) determine if mimosine can leach from defoliated leaves, (iii) determine if mimosine enhances uptake of metallic cations in giant leucaena growing in alkaline soils; and (iv) quantify the Fe-solubilizing ability of mimosine at an alkaline pH.

Materials and methods

Germination and growth of giant leucaena plants

Mature seeds of giant leucaena variety K636 were collected from the Waimanalo Research Station, University of Hawaii, on the island of Oahu, HI. Mature seeds were scarified by immersing them in concentrated H₂SO₄ for 10 min with gentle agitation at room temperature. After scarification, seeds were rinsed several times with diH₂O and germinated in 21' × 10' plastic trays containing a potting soil and vermiculite mix (50/50 v/v) (pH 5.8). Potting soil contained a heterogeneous mixture of organic and inorganic matter. Plants were watered weekly with ¼ strength Hoagland solution (pH 5.8). The seedlings were grown for one-month in a plant growth chamber at 25° C ± 2° C with a 16/8 h light/dark photoperiod with an irradiance of 100 μmol s⁻¹ m⁻², and an average humidity between 60–65%.

Collection and determination of mimosine in the rhizosphere soil surrounding giant leucaena roots

It was expected that if giant leucaena plants release mimosine into the soil, mimosine should be present in the soil surrounding its roots. Therefore, to determine the presence of mimosine in the soil, growth media containing a potting-soil-vermiculite mixture (50/50 v/v) (pH 5.8) and previously harboring giant leucaena plants were collected and pooled. Mimosine was eluted by rinsing the used soil several times with 0.1 N HCl. The ‘soil eluate’ was pooled and concentrated using a Labconco freeze dry system (Labconco, Kansas City MO, USA) and then stored at -80° C.

Mimosine in old dried plant tissues

It is possible that defoliated plant parts, both fresh or old, can leach mimosine from their tissues into the ground below. A lot of mimosine (2–5% DW) is present in fresh leucaena foliage; however, it is not known how much mimosine remains in old, dry defoliated leaves. Therefore, to determine if old and dried giant leucaena plant parts still contain mimosine that can be leached out, the leaves, stems and roots of old and dried giant leucaena (> 2 years old) were collected and further dried overnight at 65 °C. To extract mimosine, 1 g of dried tissue was submerged in 30 mL of 0.1 N HCl. The samples were incubated overnight at room temperature with shaking. After incubation, plant tissue extracts were analyzed for the presence of mimosine using HPLC. The amount of mimosine in the plant tissue was determined as a percentage of the plant dry weight. Each sample set contained at least three replications.

Mimosine release from fallen giant leucaena leaves

To determine if fresh and old dried leaves of giant leucaena can leach mimosine into the ground below, 5 g of fresh giant leucaena leaves were placed on the top of pots containing 1 kg DW of unused potting-soil-vermiculite mixture (50/50 v/v) (pH 5.8). In second experiment, 2 g of dried defoliated giant leucaena leaves were placed on the top of pots containing 1 kg DW of unused soil-vermiculite mixture. To mimic rainfall, 100 mL of diH₂O was sprinkled on the surface of the growth media every day for one-week. The soil flow-through water was collected each day for

each experimental set and then combined. The soil flow-through water experimental set was analyzed for the presence of mimosine. The amount of mimosine leached from leaves was calculated and determined as an amount (µg) of the growth media DW (g). Each sample set contained at least three replications.

Mimosine on plants growing directly below leucaena foliage

In addition to root exudate and leaching from defoliated leaves, it is possible that during periods of rain, mimosine could be washed from the surface of leucaena plant leaves to the area below. To test this, the foliage of *Vitex rotundifolia* that was growing directly below the foliage of giant leucaena, and the foliage of the same species, but growing away from giant leucaena foliage, were collected after a period of moderate rain. The leaf samples were rinsed several times in 0.1 N HCl and then the eluate analyzed for the presence of mimosine following the methods described above. Plants growing away from the leucaena foliage was used as the control. Each sample set contained at least four replications.

Collection of giant leucaena root exudates

To determine if mimosine is secreted in the giant leucaena root exudates, one-month old seedlings were transferred from potting-soil-vermiculite mixture (50/50 v/v) (pH 5.8) to hydroponic growth media containing ¼ strength Hoagland solution (pH 5.8) and grown for one-week to allow the plants to adjust to new media conditions. After one-week, the plants were transferred to fresh hydroponic media containing ¼ strength Hoagland solution (pH 5.8) and then grown for one week. Hydroponic media were collected and replaced daily. The collected media were pooled separately and then freeze dried using a Labconco freeze dry system. All samples were stored at -80° C until needed.

To determine if pH affects secretion of mimosine, one-month old giant leucaena seedlings were transferred from potting-soil-vermiculite mixture (50/50 v/v) (pH 5.8) to hydroponic media containing ¼ strength Hoagland solution (pH 5.8) and grown for one-week to allow plants to adjust to new media conditions. After one-week, the plants were transferred to fresh hydroponic media containing ¼ strength

Hoagland solution and buffered to either pH 6.0, 7.0, or 8.0 using 0.05 M MES or 0.1 M Tris–HCl and then grown for one-week. Hydroponic media were collected and replaced daily. The collected media were pooled separately for each pH and then concentrated using a Labconco freeze dry system. All samples were stored at -80°C until needed. After one week, all the plants were dried and the amount of mimosine secreted determined as a percentage of the plant dry weight. Each sample set contained four biological replications.

To determine if nitrogen availability affects secretion of mimosine, one-month old leucaena seedlings were transferred from potting-soil-vermiculite mixture (50/50 v/v) (pH 5.8) to hydroponic growth media containing $\frac{1}{4}$ strength Hoagland solution (pH 5.8) and grown for one-week to let plants adjust to new media. After one-week, leucaena plants were transferred to fresh hydroponic media containing $\frac{1}{4}$ strength Hoagland solution (pH 5.8) but no nitrogen and supplemented with either 0, 0.2, 0.8, or 3.2 mM ammonium nitrate and then grown for two-weeks. Hydroponic media were collected and replaced daily. The collected media were pooled separately for each concentration of ammonium nitrate and then concentrated using a Labconco freeze dry system. All samples were stored at -80°C until needed. After one-week, all plants were dried and the amount of mimosine secreted determined as a percentage of the plant dry weight. Each sample set contained four biological replications.

High performance liquid chromatographic analyses of old dried giant leucaena tissue extracts, rhizosphere soil eluate, root exudates, and leaf wash of plants growing directly below giant leucaena foliage

All soil eluates and root exudates samples were diluted with diH_2O and filtered to remove insoluble material. Mimosine in the samples was determined with a Waters 2695 high performance liquid chromatography (HPLC) system equipped with a photodiode array detector (Waters, Millford, MA, USA) and a Phenomenex C18 column (Phenomenex, Torrance, CA, USA). Mimosine was eluted with 0.02 M o-phosphoric acid aqueous solution at a constant flow rate of 1 mL/min and detected at $A_{280}\text{ nm}$. A mimosine authentic standard (Millipore-Sigma, St. Louis,

MO, USA) was used to identify mimosine and calibrate concentrations.

Liquid chromatography/mass spectrometry (LC/MS) analyses of rhizosphere soil eluate and root exudates

To confirm the presence of mimosine in the soil eluate, and in the root exudates, rhizosphere soil eluates and root exudates were collected, pooled and stored in the -80°C . Sample preparation methods can be found in the supp material (S1). Mimosine in the lyophilized samples was analyzed on an Agilent 6410 Triple Quadrupole LC/MS (Agilent Technologies, Santa Clara, CA, USA). The LC/MS was operated at electrospray ionization (20 eV) and the negative MRM mode to detect ions of mass to charge ratio (m/z) of 197.1 (M-H) and the product ion m/z 110.0 (hydroxy-pyridone ring of mimosine).

Mimosine supplied to leucaena plants growing in alkaline soils

Metal concentrations

To determine if mimosine supplied to plants enhances uptake of Fe, giant leucaena seedlings were grown in alkaline soil (pH 8.0–8.5) collected from the Waimanalo Research station. The Fe concentration of soil was previously determined to be $\sim 1.98 \pm 0.05\ \mu\text{g/g DW}$ (extractable soil Fe). Plants were grown for one-month and supplied with either water only, water+100 μM EDDHA, or water+100 μM mimosine. All solutions were adjusted to pH 8.0–8.5 using NaOH. After one-month, whole plants (leaves, stems, and roots) were harvested, dried and ground into powder using mortar and pestle. Samples were sent to the College of Tropical Agriculture and Human Resources Agricultural Diagnostic Services Center (CTAHR ADSC) for determination of metal concentration in whole plant tissues by ICP-OES. Each sample set contained at least four biological replications.

Chlorophyll concentration

Giant leucaena seedlings were grown in alkaline soil for one-month and supplied with either water only, water+100 μM EDDHA, or water+100 μM mimosine. After one-month, 50 mg of fresh leaflets were collected

from plants and then chlorophyll extracted in a non-destructive method using modified methods described by Richardson et al. (2002) and Siebeneichler et al. (2019). In this method, 50 mg of fresh leaves was submerged in 3 mL of dimethyl sulfoxide (DMSO) and heated at 65° C for 30 min. The DMSO extract was removed and transferred to a separate tube and stored at 4° C. This process was repeated until leaflets were transparent. The absorbances of DMSO extracts were assessed at 470, 645, and 663 nm using pure DMSO as the blank. Chlorophyll concentrations were quantified and determined as a % of plant fresh weight (FW) using the equations from Siebeneichler et al. (2019). Each sample set contained ten replications.

Iron solubilizing properties of mimosine

An amount of 50 mg of goethite or hematite was weighed and placed in 10 mL centrifuge tubes containing either diH₂O (control), diH₂O + 1 mM EDDHA, diH₂O + 1 mM mimosine, diH₂O + 2 mM mimosine, or diH₂O + 3 mM mimosine. All samples were adjusted to pH 8.0 using 0.1 M Tris–HCl. Samples were then mixed for 1 h at room temperature and centrifuged at 5000×g for 10 min. The soluble fractions were then collected and sent to the CTAHR ADSC for determination of Fe concentration by ICP-OES. Each sample set contained six replications.

Fe(III)-mimosine complexes were prepared at 1:0, 1:1, 1:2 and 1:3 molar ratios using FeCl₃. Fe(III)-EDDHA was also prepared at 1:1 molar ratio. All samples were adjusted to pH 8.0 using 0.1 M Tris–HCl. Samples were then mixed for 1 h at room temperature and centrifuged at 16,000×g for 5 min. The soluble fractions were collected and sent to the CTAHR ADSC for determination of Fe concentration by ICP-OES. Each sample set contained six replications.

Statistical analysis

An analysis of variance (ANOVA) test followed by a Tukey post-hoc test was used for data analysis.

Results

Mimosine in the rhizosphere soil and root exudates of giant leucaena

Mimosine in the rhizosphere soil eluates and giant leucaena root exudates was identified by comparison

with the standard mimosine, at a retention time of 2.7 min (Fig. 1). The identities were confirmed by LC/MS MRM analyses (Figs. 2 and 3). A fragment at a *m/z* of 110.1, corresponded to the hydroxy-pyridone group of mimosine ([M] + -H, *m/z* = 197.1). The difference in the retention times of HPLC and MS/MS data are due to the different types of solvent and column used and, the day to day differences in method development. These results indicate that giant leucaena roots release mimosine into the rhizosphere.

Mimosine in old dried plant tissues

The extracts of old dried leaves, stems and roots all had peaks at 2.7 min indicating mimosine was present in the giant leucaena tissues (Fig. 4a–c). The old dry

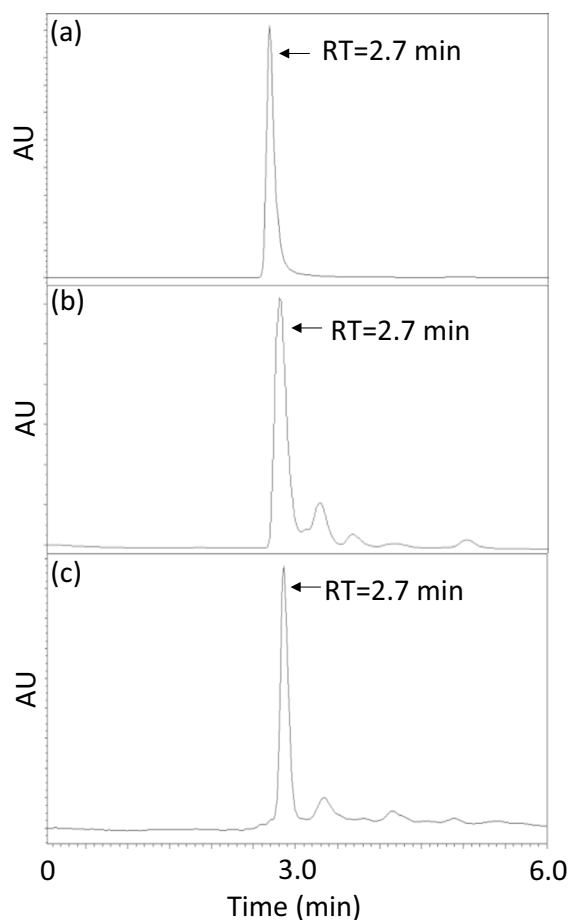


Fig. 1 HPLC chromatograms of (a) commercial mimosine (standard), (b) giant leucaena rhizosphere soil eluate, and (c) giant leucaena root exudate

Fig. 2 MS1 mass chromatogram of (a) commercial mimosine standard and MS2 multiple reactions monitoring (MRM) chromatograms of (b) fragmented commercial mimosine (standard). MS1 mass chromatogram of (c) HPLC purified mimosine in the giant leucaena root exudates and MS2 multiple reactions monitoring (MRM) chromatograms of (d) fragmented mimosine in the giant leucaena root exudate

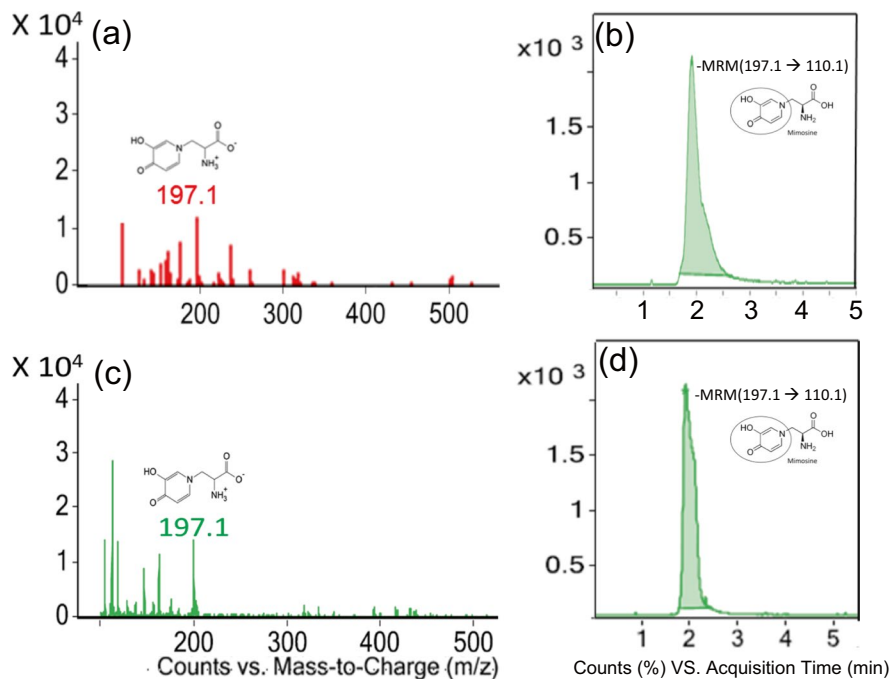
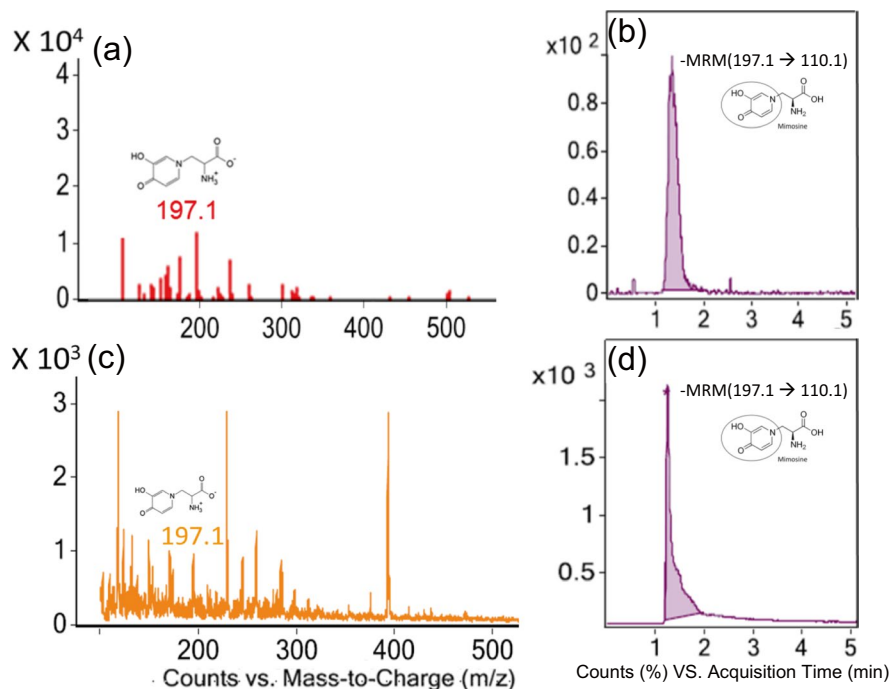


Fig. 3 MS1 mass chromatogram of (a) commercial mimosine standard and MS2 multiple reactions monitoring (MRM) chromatograms of (b) fragmented commercial mimosine (standard). MS1 mass chromatogram of (c) HPLC purified mimosine in the giant leucaena rhizosphere soil eluate and MS2 multiple reactions monitoring (MRM) chromatograms of (d) fragmented mimosine in the giant leucaena rhizosphere soil eluate



leaves contained 0.16% DW mimosine. Roots had the lowest amount of mimosine relative to leaves, which had twofold more mimosine than the roots (Fig. 4d). These results suggest that mimosine remains stable inside old plant parts.

Mimosine release from fallen giant leucaena leaves

Both sets of soil eluates (old dried and fresh leaves) produced a peak at 2.7 min indicating that mimosine was leaching from leaves into the soil below (Fig. 5).

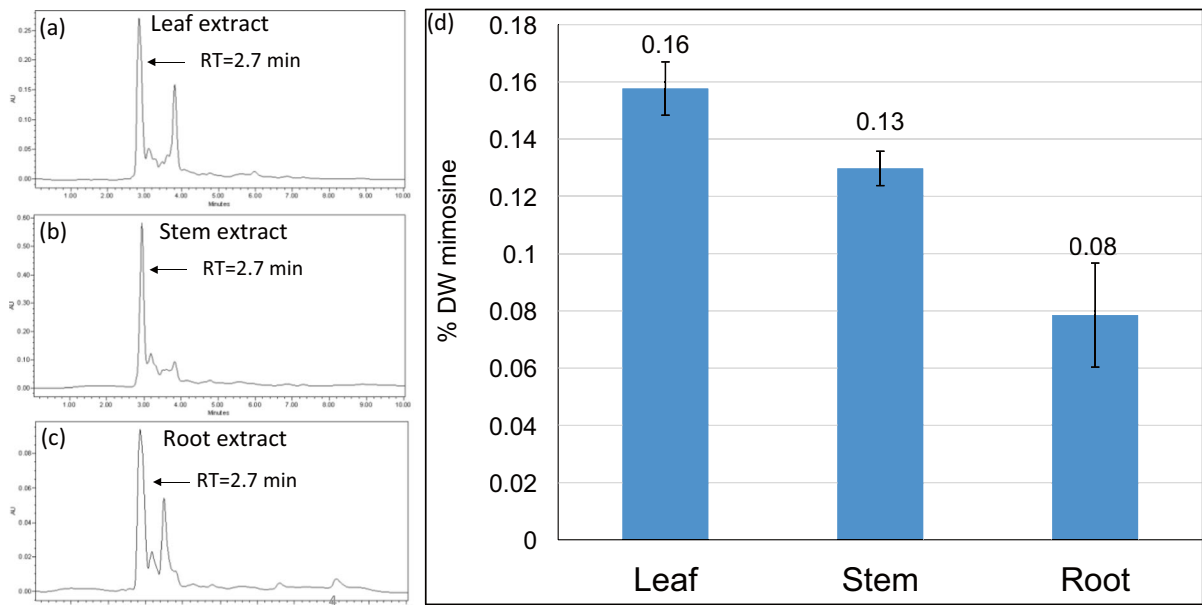


Fig. 4 HPLC chromatograms of old dried (a) leaf extract, (b) stem extract, and (c) root extract, and (d) mimosine concentrations within old dried fallen leaf, stems and roots. Error bars indicate standard error

The amount of mimosine that leached from old, dry and defoliated giant leucaena leaves were found to be 1.15 ± 0.22 $\mu\text{g/g}$ of soil DW when fresh leaves were placed on the top of the soil and water applied, and 0.54 ± 0.38 $\mu\text{g/g}$ soil DW when old dried leaves were used.

Mimosine secretion at pH 6.0, 7.0, and 8.0

The giant leucaena plants grown at pH 8.0 by far secreted the most mimosine, followed by those grown at pH 7.0 and 6.0 (Fig. 6). These results indicate that more mimosine was secreted in the root exudates when the plants were grown at an alkaline pH.

Mimosine secretion at different levels of combined nitrogen

Giant leucaena plants supplied with increasing amounts of ammonium nitrate secreted much more mimosine than those not supplied with nitrogen (Fig. 7). The results indicate a positive correlation between the amounts of mimosine secreted and nitrogen source provided to the plants.

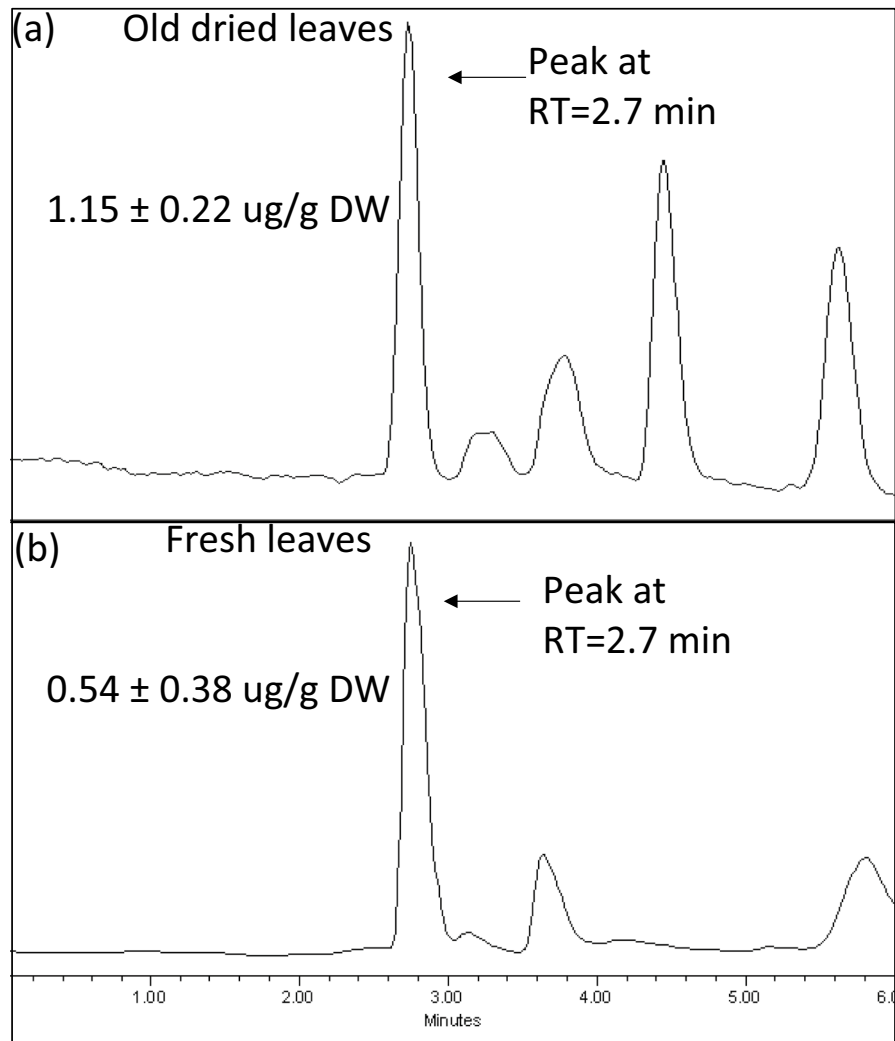
Mimosine on plants growing directly below giant leucaena foliage

The leaf wash of plants not growing directly beneath leucaena plants served as the negative control and did not produce a peak at 2.7 min. The leaf wash of plants growing directly below leucaena foliage had peaks at 2.7 min indicating that mimosine was present on the leaf surface of these plants (Fig. 8). These results indicate that mimosine was transferred from giant leucaena foliage to the surface of leaves of plants growing understory.

Metal concentration of giant leucaena

Giant leucaena plants supplied with mimosine had higher Fe and Mn concentrations than the control plants (Fig. 9). Interestingly, giant leucaena plants supplied with mimosine also had lower concentrations of the heavy metal's Cr and Cd. Not surprisingly, plants supplied with EDDHA also had higher Fe, Mn, Ni, Cu, Pb, and Co and concentrations than control plants. The results suggest that mimosine supplied to giant leucaena, enhances Fe uptake from alkaline soils.

Fig. 5 HPLC chromatograms of mimosine in the soil eluate of pots containing soil-vermiculite mixture when (a) old dried leaves or (b) fresh leaves were placed on top of the mixture in pots



Chlorophyll concentration of giant leucaena

Giant leucaena plants supplied with mimosine had higher total chlorophyll concentrations than plants supplied with just water (Fig. 10). Plants supplied with EDDHA had higher total chlorophyll concentrations than control plants. The results suggest that mimosine supplied to giant leucaena plants enhances chlorophyll synthesis.

Mimosine and solubility of Fe oxides

Mimosine significantly enhanced extraction of Fe from goethite and hematite (Fig. 11a and b).

Interestingly, mimosine bound to ferric ions at pH 8.0, was able to limit the conversion of ferric to insoluble ferric oxides (Fig. 11c). The results suggest that mimosine can extract and solubilize Fe from insoluble mineral oxides. These results also suggest that mimosine can prevent the conversion of ferric ions to insoluble ferric oxides.

Discussion

Phytosiderophores such as DMA and nicotianamine (NA), are known to be secreted in the root exudates of plants, and to be involved in the uptake of Fe

Fig. 6 Amounts of mimosine secreted in giant leucaena root exudates when plants were grown in media at pH 6.0, 7.0, and 8.0. Error bars indicate standard error of four replicates. Bars sharing a letter of the same case do not differ by Tukey post-hoc test ($P < 0.05$)

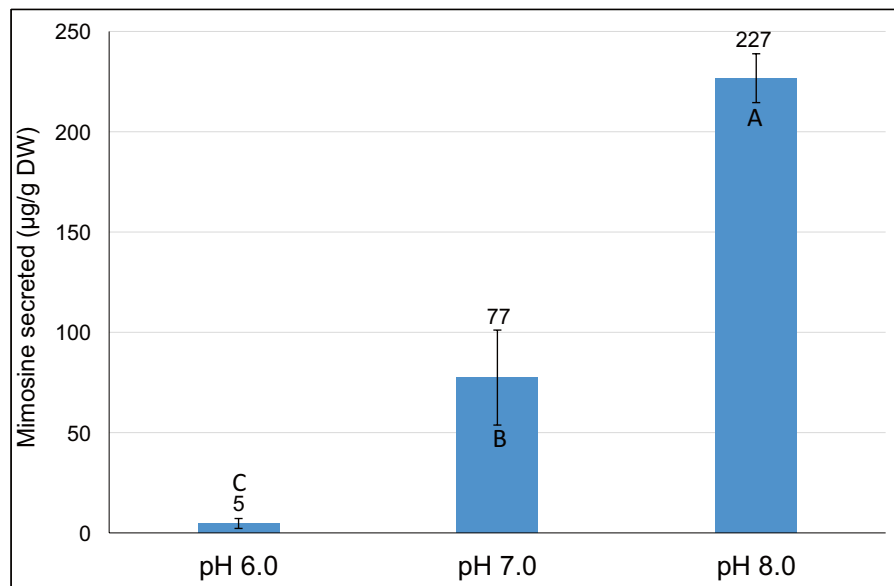
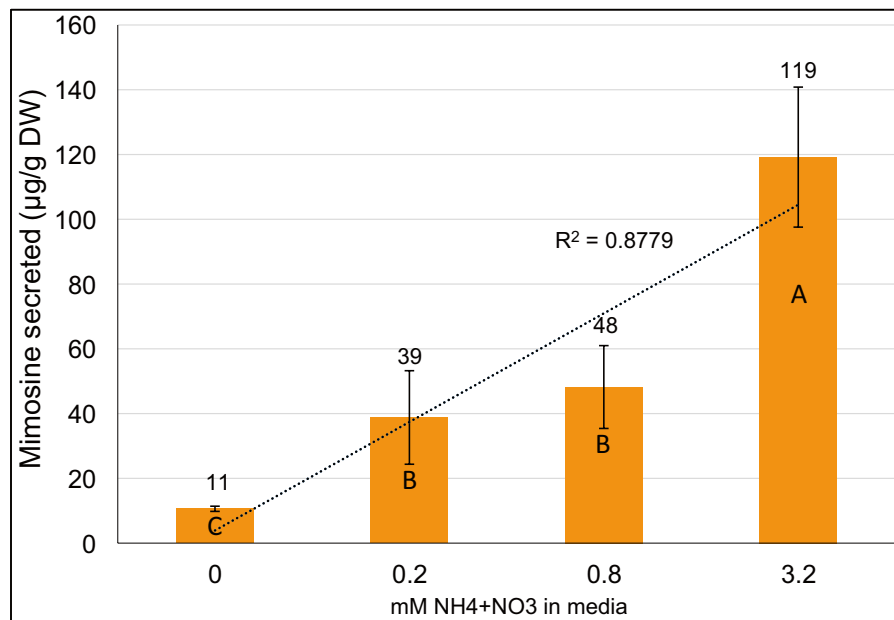


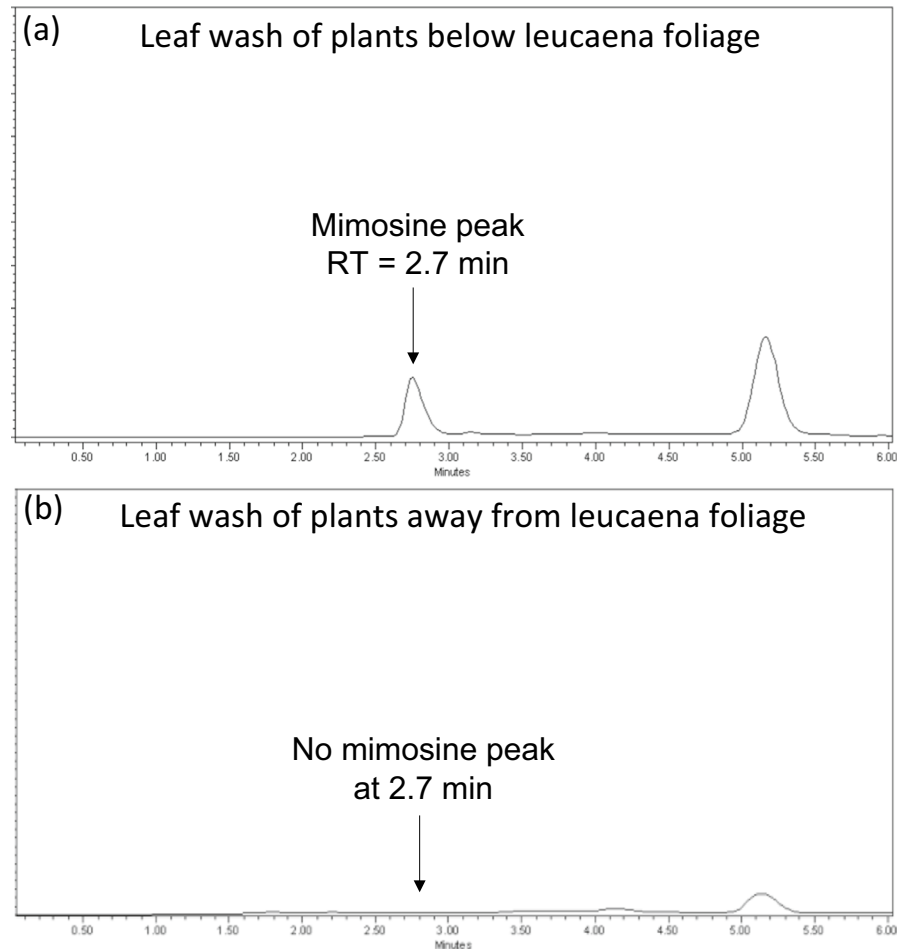
Fig. 7 Mimosine secretion by giant leucaena roots when increasing amounts of nitrogen were supplied to plants. Error bars indicate standard error of four replicates. Bars sharing a letter of the same case do not differ by Tukey post-hoc test ($P < 0.05$)



from the soil and its transport within plants (Suzuki et al. 2006; Tsednee et al. 2014). Phytosiderophores increase the solubility of soil Fe through chelation and formation of Fe-phytosiderophore complexes (Kobayashi et al. 2014; Roberts et al. 2004). In a study conducted by Walter et al. (2017), it was found that Fe mobilization by DMA was increased by application of lime to soils. Oburger et al. (2014) found that DMA was secreted at significantly different rates

by wheat plants growing in calcareous soils. DMA is known to be released under Fe deficiency conditions (Cakmak et al. 1994; Gries et al. 1995; Ma 2005). In the present study, it was found that root secretion of mimosine by giant leucaena was highest when plants were grown under alkaline conditions. When Fe is less soluble and sparingly available to plants, it was found that mimosine binds to Fe(III) best at alkaline pH 8.0 and 8.5 (Honda and Borthakur 2020). In the

Fig. 8 HPLC chromatograms of leaf wash for *V. rotundifolia* plants located directly (a) below and (b) away from giant leucaena foliage after a rain shower



same study, it was shown that mimosine-Fe complexes are hydrophilic under alkaline conditions, and that mimosine was able to extract Fe directly from alkaline soils. Previously, Soedarjo and Borthakur (1998) observed that mimosine was released in the root exudates of leucaena plants. They also found that mimosine secretion and the mimosine concentration of leaves were higher in *Rhizobium*-inoculated plants. However, these results were not confirmed by LC/MS analysis. In the present study, LC/MS analyses confirmed the presence of mimosine in the giant leucaena root exudates and in the soils surrounding giant leucaena roots. It was also found that secretion of mimosine was higher when giant leucaena plants were supplied with increasing amounts of nitrogen. It is known that the mimosine concentration of giant leucaena tissues increases with higher availability of nitrogen (Honda and Borthakur 2021). Mimosine contains two nitrogen molecules, and therefore, it is

not surprising that supplying plants with exogenous nitrogen sources, or inoculating plants with nitrogen-fixing *Rhizobium* increases both the mimosine concentration of plants and the secretion of mimosine in the root exudates.

In strategy I plants, phenolic compounds are known to be secreted in plant root exudates in response to Fe deficiency conditions (Jin et al. 2007). Specifically, secretion of phenolic coumarins have been shown to be increased from *Arabidopsis thaliana* roots in response to Fe deficiency at increased pH (Sisó-Teraza et al. 2016b). Secretion of phenolic coumarins have been shown to be important in tolerance of *A. thaliana* to Fe deficiency at elevated pH (Schmid et al. 2014; Schmidt et al. 2014). In the present study, secretion of mimosine in root exudates was found to be increased in giant leucaena plants that were grown at increasing pHs. Like other phenolic compounds, mimosine secretion is increased under Fe-limiting

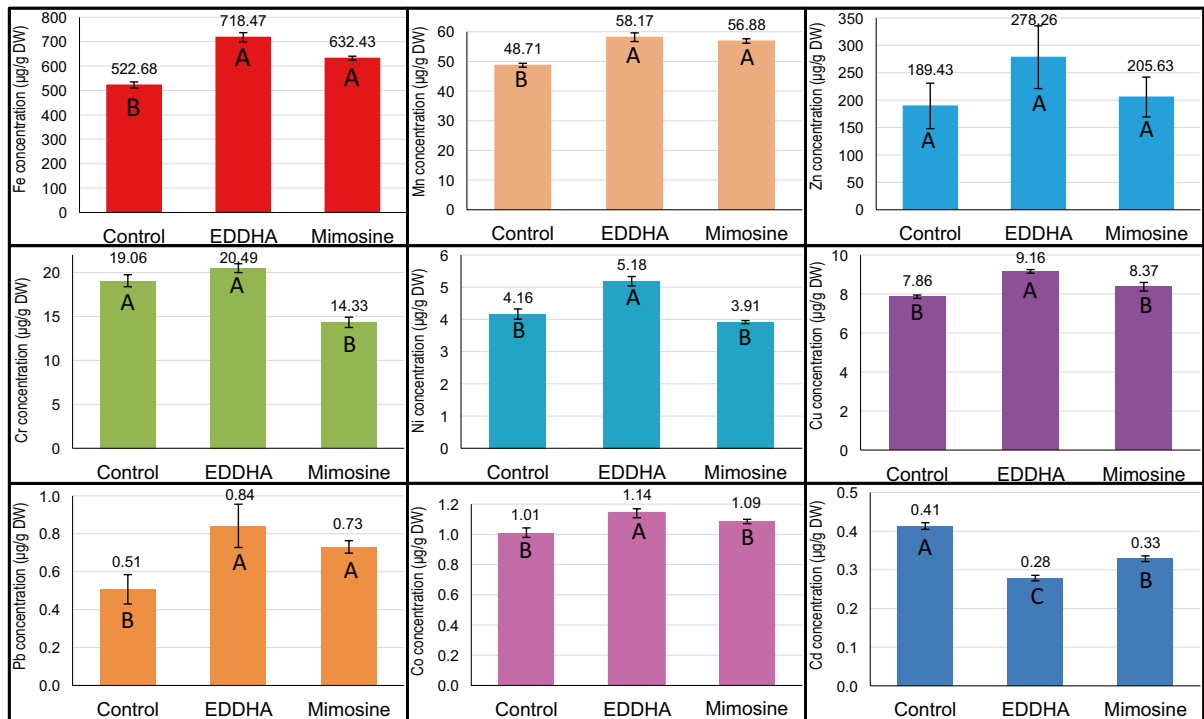


Fig. 9 Metal concentrations of giant leucaena whole plants that were grown in alkaline soil (pH 8.0–8.5) for one month and supplied weekly with either water (control), EDDHA, or

mimosine. Error bars indicate standard error of four replicates. Bars sharing a letter of the same case do not differ by Tukey post-hoc test ($P < 0.05$)

conditions, such as alkaline pH. In another study conducted by Sisó-Terraza et al. (2016a), it was found that flavins secreted by *Beta vulgaris* improved Fe

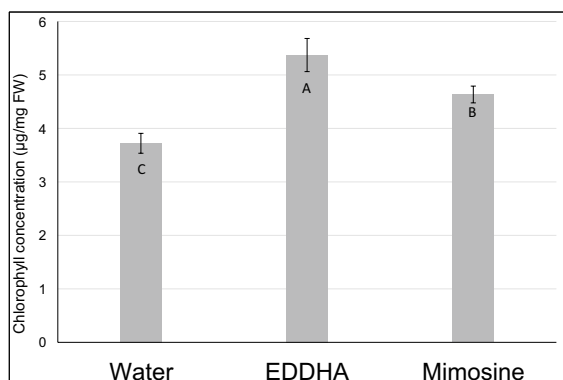


Fig. 10 Total chlorophyll concentration of giant leucaena plants grown in alkaline soil for one month and supplied with either water (control), EDDHA, or mimosine. Error bars indicate standard error of ten replicates. Bars sharing a number or a letter of the same case do not differ by Tukey post-hoc test ($P < 0.05$)

nutrition. They also found that flavins enabled *B. vulgaris* to mine Fe from ferric-oxides through reduction. Honda and Borthakur (2020) found that *Phaseolus coccineus* supplied with various Fe-mimosine complexes had higher Fe concentrations than plants supplied with only Fe. They also found that plants supplied with Fe-mimosine complexes accumulated more mimosine than plants supplied with only mimosine, suggesting that Fe-mimosine complexes were taken up by *P. coccineus* plants. In the present study, giant leucaena plants growing in alkaline soil and supplied with mimosine had increased Fe concentrations. Similar to secreted flavins, mimosine also increased Fe mobilization in giant leucaena, which may also be due to Fe mining or through reduction of Fe-oxides. In the present study, mimosine was found to solubilize Fe from the insoluble Fe mineral oxides goethite and hematite at an alkaline pH. It was also found that mimosine limited the formation of insoluble Fe-oxides at pH 8.0. Enhancing the solubility of soil Fe by mimosine could explain why leucaena is well-adapted to alkaline soils.

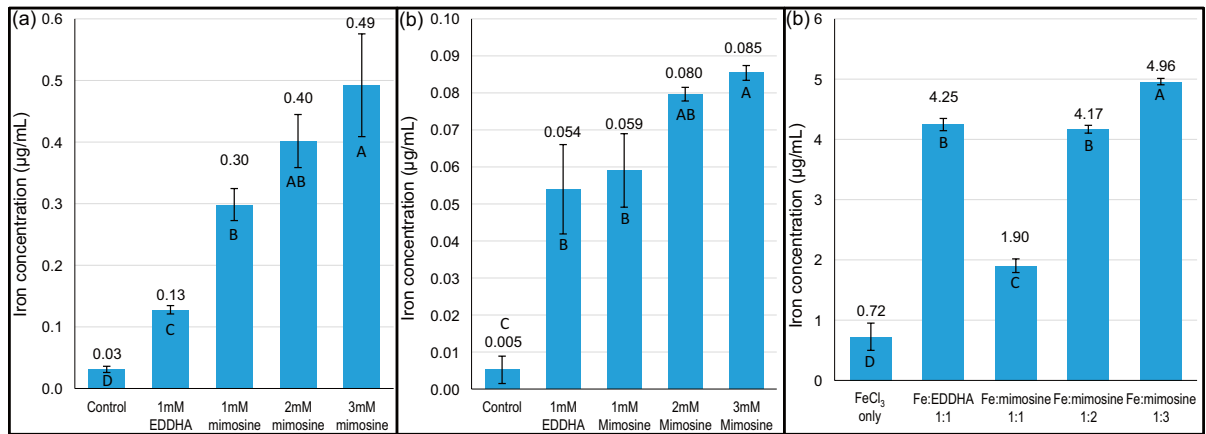


Fig. 11 Iron concentrations of the soluble/water phase of samples containing (a) goethite, (b) hematite, and (c) FeCl₃ at pH 8.0. Error bars indicate standard error of six replicates. Bars sharing a letter of the same case do not differ by Tukey test ($P \leq 0.05$)

In the present study, giant leucaena plants growing in alkaline soils and supplied with mimosine had higher Fe and Mn concentrations than control plants. The presence of mimosine facilitated Fe and Mn uptake, however, not Cu, Co or Zn. The ability of mimosine to extract and solubilize Fe from the alkaline soil is the likely reason for the increase in Fe concentrations in whole plant tissues. The increased Fe solubility leads to increased Fe uptake by giant leucaena plants. Mimosine can bind Fe in at least three different ways, and has been suggested to form polynuclear metal ligand complexes with Fe (Honda and Borthakur 2020). Citrate is also known to form polynuclear species with Fe (Silva et al. 2009). The formation of polynuclear metal–ligand species may increase the amount of Fe that is solubilized and made available to plants. Besides increasing the solubility and availability of soil Fe, it is possible that mimosine chelated to Fe may serve as a substrate for FRO enzymes that reduce Fe(III) to Fe(II). Iron-citrate and Fe-malate complexes have been shown to be substrates for leaf FRO enzymes (Gonzalez-Vallejo et al. 1999). Citrate is secreted in the root exudate of plants and secretion has been shown to be increased in plants under Fe deficiency (Tiffin 1966a, b). Citrate can bind Fe under physiological conditions (Silva et al. 2009). It is possible that mimosine, which is a bidentate ligand, could serve as a substrate for FRO when bound to Fe in its various forms.

Giant leucaena can release mimosine into the soil utilizing multiple mechanisms. Mimosine can be

secreted in the root exudate, leach from fresh and old defoliated leaves and tissues, and can be washed from the leaf surface by rain. Honda and Borthakur (2019) found that dried leucaena leaves still contained mimosine. Similarly, the present study found that old dried leucaena plant parts still contained considerable amounts of mimosine, which could leach from these tissues into the soil. The various ways mimosine is released by giant leucaena into the ground would explain the presence of mimosine in the rhizosphere soil eluate. The mimosine released into the ground may serve to extract Fe from the soil and make it more abundant and available to plant roots. Defoliated tissues may provide a constant reservoir of mimosine that is released during rain. The ability of mimosine to extract and solubilize Fe at alkaline pH, its presence in the soil, and the multiple mechanisms of mimosine release into the soil, can explain the adaptability of giant leucaena to alkaline soils, where Fe is less abundant and available to plants.

Conclusion

Mimosine is released by giant leucaena into the rhizosphere soil through root exudates. Secretion of mimosine was the highest when giant leucaena was grown under alkaline conditions. Giant leucaena plants supplied with mimosine had higher Fe concentrations

than the control plants. The increase in Fe concentration in giant leucaena was likely due to enhanced Fe solubility and uptake. Mimosine can solubilize Fe from two common forms of Fe in the soil, goethite and hematite, both of which being insoluble Fe oxides. Mimosine is also able to limit the formation of insoluble ferric-oxides under an alkaline pH. The ability of mimosine to enhance Fe solubility may be a major factor for giant leucaena's tolerance to alkaline soils.

Author contribution Experiments for this manuscript were conducted by MDHH with discussions with DB. Diagrams, images, and tables were created by MDHH. The manuscript was written by MDHH in discussion with DB. The document was reviewed and edited by DB.

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